Promega

Application Note

Measuring the QuantiFluor™ dsDNA System Using the QuantiFluor™-ST Fluorometer

INTRODUCTION

Accurate quantitation of DNA is critical for many applications. Traditional spectrophotometric assays cannot determine DNA concentration below 2µg/ml; however, many isolated DNA samples have concentrations well below that level. The QuantiFluor™ dsDNA System (Cat.# E2670) provides a fast, easy and sensitive method for determining DNA concentrations as low as 2.5ng/ml (or 0.5ng/well). The QuantiFluor™ dsDNA System contains a fluorescent DNA-binding dye that enables sensitive and specific quantitation of small amounts of double-stranded DNA (dsDNA) in solution. The dye shows minimal binding to single-stranded DNA (ssDNA) and RNA, allowing specific quantitation of dsDNA.

This Application Note describes the protocol for using the QuantiFluor™ dsDNA System with the QuantiFluor™-ST Fluorometer and the PCR Tube Adapter. The QuantiFluor™ PCR Tube Adapter allows sample volumes as little as 100µl without sacrificing instrument sensitivity.

MATERIALS REQUIRED

- QuantiFluor™ dsDNA System (Cat.# E2670)
- QuantiFluor™-ST Fluorometer (Cat.# E6090 or E6105)
- PCR Tube Adapter, QuantiFluor™ Fluorometers (Cat.# E6101)
- 0.5ml PCR tubes (Axygen Cat.# PCR-05-C, available through Fisher or VWR)

Caution: We recommend use of gloves, lab coats and eye protection when working with these or any chemical reagents.

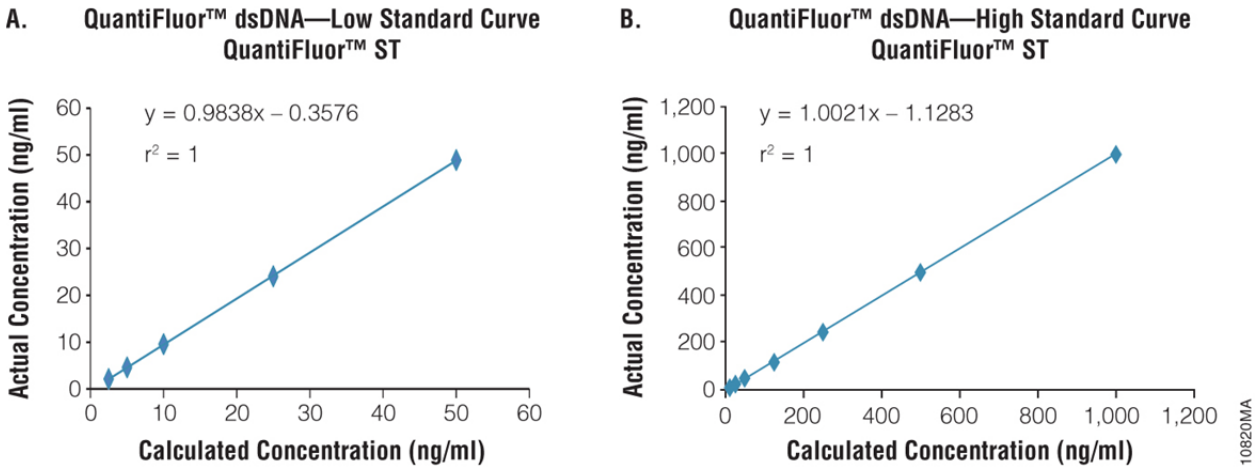

Figure 1. Measuring dsDNA concentration using the QuantiFluor™ dsDNA System and the QuantiFluor™-ST Fluorometer with PCR Tube Adapter. Panel A. Assay linearity using the low standard curve. **Panel B.** Assay linearity using the high standard curve.

EXPERIMENTAL PROTOCOLS

A. For High-Concentration DNA Samples (10–1,000ng/ml or 2–200ng per tube)

1. Dilute the QuantiFluor™ dsDNA Dye 1:200 in 1X TE buffer to make a working solution. For example, add 10µl of QuantiFluor™ dsDNA Dye to 1,990µl of 1X TE, and mix.
2. Add 100µl of the QuantiFluor™ dsDNA Dye working solution to an empty 0.5ml PCR tube. This will be the Blank used in Section C, Step 7. Protect from light.
3. Dilute the DNA standard 1:50 in 1X TE buffer to a concentration of 2ng/µl. For example, add 20µl of DNA Standard to 980µl of 1X TE, and mix.
4. Add 100µl of the diluted DNA Standard and 100µl of the QuantiFluor™ dsDNA Dye working solution to a 0.5ml PCR tube and mix. This will be the Standard used in Section C, Step 9.
5. Add 100µl of the unknown sample and 100µl of the QuantiFluor™ dsDNA Dye Working Solution to a 0.5ml PCR tube, and mix.
   Note: If the volume of the unknown DNA sample is less than 100µl, add 1X TE buffer to a final volume of 100µl. Record the volume of unknown DNA sample added per tube. This dilution factor will be used later to calculate the final DNA concentration in ng/ml.
6. Incubate the Standard and unknowns at room temperature for 5 minutes, protected from light.

B. For Low-Concentration DNA Samples (2.5–50ng/ml or 0.5–10ng per tube)

1. Dilute the QuantiFluor™ dsDNA Dye 1:200 in 1X TE buffer to make a working solution. For example, add 10µl of QuantiFluor™ dsDNA Dye to 1,990µl of 1X TE, and mix.
2. Add 100µl of the QuantiFluor™ dsDNA Dye working solution to an empty 0.5ml PCR tube. This will be the Blank used in Section C, Step 7. Protect from light.
3. Dilute the DNA Standard 1:1000 in 1X TE buffer to a concentration of 0.1ng/µl. For example, add 1µl of DNA Standard to 999µl of 1X TE, and mix.
4. Add 100µl of the diluted DNA Standard and 100µl of the QuantiFluor™ dsDNA Dye working solution to a 0.5ml PCR tube and mix. This will be the Standard used in Section C, Step 9.
5. Add 100µl of the unknown sample and 100µl of the QuantiFluor™ dsDNA Dye Working Solution to a 0.5ml PCR tube, and mix.
   Note: If the volume of the unknown DNA sample is less than 100µl, add 1X TE buffer to a final volume of 100µl. Record the volume of unknown DNA sample added per tube. This dilution factor will be used later to calculate the final DNA concentration in ng/ml.
6. Incubate the Standard and unknowns at room temperature for 5 minutes, protected from light.

C. Setting Up the QuantiFluor™-ST Fluorometer

1. Insert the PCR Tube Adapter into the QuantiFluor™-ST Fluorometer.
   Note: The PCR Tube Adapter is multidirectional and can be inserted in any orientation.
2. Press the **ON/OFF** button to turn the instrument on.
3. Set the instrument to the Blue channel by pressing the **A/B** button. The display should read "BLUE".
4. Set the standard value by pressing the **STD VAL** button.
   a. If using the **High Standard Dilution**, set the Instrument standard to **200 (ng per tube)**.
   b. If using the **Low Standard Dilution**, set the Instrument standard to **10 (ng per tube)**.
5. Press the **CAL** button; the screen will display "Calib BLUE <ENT> to start".
6. Press the **ENTER** button to move to the next screen, which will display "Insert Blank then press <ENT>".
7. Insert the blank sample, and press the **ENTER** button. The QuantiFluor™-ST Fluorometer will calculate the average reading over 10 seconds and set the zero (blank) point. During this time the screen will display "Reading Blank".
8. After the instrument has set the zero point, the screen will display "Insert Cal Soln then press <ENT>".
9. Insert your DNA standard (from Section A, Step 4, or Section B, Step 4), and press the **ENTER** button. The instrument is now calibrated.
10. Insert an unknown DNA sample. Press the **READ** button. The instrument will display the concentration of DNA in ng/tube. If 1µl of sample was added in Section A, Step 5, or Section B, Step 5, then the value displayed is equal to the concentration in ng/µl. If 2µl were added, then divide the displayed value by 2 to calculate the concentration in ng/µl of the original undiluted unknown. If 5µl were added, divide the displayed value by 5 to calculate the concentration in ng/µl of the original undiluted unknown. To convert from ng/µl to ng/ml, multiply by 1,000.

CONTACT INFORMATION

Toll-Free: (800) 356-9526
Fax: (800) 356-1970

www.promega.com

Email: Custserv@promega.com for ordering inquiries
Email: Techserv@promega.com for technical inquiries

Mailing Address:

Promega Corporation
2800 Woods Hollow Rd.
Madison, WI 53711 USA